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CONTENTS

NUZUM, M. F., AND RAND, H. W.	*Can the Earthworm Pharynx Epithelium Produce Central Nervous Tissue?*	213
WOODBRIDGE, HELEN.	*Botryllus schlosseri (Pallas): The Behavior of the Larva with Special Reference to the Habitat*	223
COPELAND, M., AND WIEMAN, H. L.	*The Chemical Sense and Feeding Behavior of Nereis virens Sars*	231
KEPNER, W. A., AND BARKER, J. F.	*Nematocysts of Microstoma*	239
BILLS, CHARLES E.	*Some Effects of the Lower Alcohols on Paramecium*	253

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Vol. XLVII *October, 1924* *No. 4*

BIOLOGICAL BULLETIN

CAN THE EARTHWORM PHARYNX[1] EPITHELIUM PRODUCE CENTRAL NERVOUS TISSUE?

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It has long been known that, when several segments of the head end of an earthworm are removed, within a comparatively short time a new head will be regenerated. The first of the new tissues to be differentiated is the nervous tissue. Hescheler in 1898 described in detail the regeneration of the nervous and other organs in several species of earthworm. He believed the mass of "Regenerationsgewebe" from which the nerve cells differentiated to be largely of epidermal origin. This would seem to be the natural source of nerve material, since in the embryo the nervous system is formed by a thickening and infolding of the ectoderm.

The operation in which several head segments are removed involves loss to all of the tissues of that region. Consequently regeneration is an extensive process in which all of the injured tissues must be more or less concerned. But if the cephalic ganglia,

[1] The region of the alimentary canal referred to here and throughout the paper is so precisely at the junction of the buccal cavity and the muscular pharyngeal region as to occasion doubt whether its epithelium should be designated as buccal or pharyngeal. It is very slightly anterior to the heavy dorsal muscle of the pharynx. Transverse sections through the brain fundament of worms regenerating after an operation of the kind described in this paper invariably include the "Schlundcommissuren," and the "Schlundganglien" or "Schlundgeflecht" (Vejdovsky, 1884; and other authors) consisting of heavy ganglionated nerves passing from the commissures to the wall of the alimentary tube. "Schlund," at least for the Lumbricidæ, must have the sense of pharyngeal. The term "circum-esophageal commissures," commonly used in English texts, is inappropriate for Lumbricidæ because the commissures are remote from the esophagus. Vejdovsky (1884, p. 81) says: "Die Schlundcommissuren umgreifen den Pharynx. . . ." In view of these facts we designate the region in question as pharyngeal rather than buccal.

or "brain," be removed through a small incision in the body wall, the operation being carried out so that no tissue other than nervous is removed and with a minimum of damage to non-nervous tissues, the resulting regenerative situation is much simpler. The non-nervous tissues will require only a direct healing of the wounds caused by the incision. If, under these circumstances, the brain is regenerated, what will be the source of its material?

Friedländer (1895) carried out operations of the latter sort. He regarded the regenerated supra-esophageal ganglia as derived mainly from the old nerve tissue, but perhaps partly from cells of the wound tissue or "Regenerationsgewebe." He suggested that these latter cells might be of leucocytic origin. Hübner (1902) repeated the experiment and concluded that leucocytes played little or no part in the regeneration and that the epidermis was mainly responsible for it. His figures show a mass of cells, with small deeply stained nuclei, lying below the epidermis in the region of the wound and extending down to the region of the developing brain. But his figures and his scanty description do not afford convincing evidence that these cells are epidermal in origin and are actually contributing to the new brain fundament. Some time ago the senior author found, in sections of earthworms regenerating after an operation of this kind, indications that the pharynx[1] epithelium might contribute cells to the regeneration of the nervous organs of the head. Accordingly the study described below was undertaken, partly for the purpose of obtaining more complete data concerning the histogenesis of the brain following an operation in which no head segments are removed, and especially with a view to ascertaining whether pharynx epithelium can produce nerve cells. Involved in the latter question is another—will an epithelium which is not itself injured become active and participate in regeneration?

A wound is supposed to supply the stimulus for regeneration. Therefore, with reference to the question as to whether an uninjured epithelium will engage in regeneration, the ideal operation would have been to remove the brain without causing any other injury to the worm. This being obviously impossible, the following plan was devised. After narcotizing the worm in 0.2 per cent. chloretone for ten minutes, an incision was made in the dorsal body

wall, in or near the median plane, and extending from the seventh segment forward to the third. The remainder of the operation was performed under a compound dissecting microscope. Very fine oculists' scissors were used. The body wall was pinned back, exposing the cephalic ganglia lying just above the pharynx in the third segment. With dissecting needles the ganglia could be raised from the tissue below and then snipped off at either side. The operation offered considerable difficulty, so that sometimes a part of either connective with the heavy nerve which runs to the pharynx wall was removed. When this happened it was impossible to tell whether or not the pharynx was injured, but it is very likely that it was. Therefore, to obviate possible injury to the dorsal region of the pharynx wall, and in order that the epidermal wound be as remote as possible from the expected (dorsal) site of the brain anlage, the operation was in some cases modified by making the incision dorso-laterally or even sometimes ventro-laterally.

Operations of the kind described above were performed on about eighty worms. The species *Allolobophora fœtida* was used because of its capacity for quick regeneration. At various stages the operated worms were fixed in Bouin's picro-formalin, the anterior ends cut transversely, and the sections stained with hæmatein-eosin. The following description is based on a careful study of twenty-five cases.

To insure appreciation of conditions in the operated animals, reference must be made to certain features of the brain region of the normal earthworm. Fig. 1 represents diagrammatically a typical cross-section through the brain region of the normal worm. The epidermis (*e*) consists of fairly large columnar cells having oval nuclei each containing a prominent nucleolus, and among these cells are numerous large gland cells. Beneath the epidermis is the body-wall muscle (outer circular and inner longitudinal fibers) and between the inner muscle layer and cephalic ganglia (*br.*) is a space containing some connective tissue and blood vessels (*bl.v.*). Around the brain is a distinct connective tissue sheath (*c.t.s.*). The periphery of the brain tissue is largely made up of typical pear-shaped nerve cells, while the central mass is mainly fibrillar. Continuing from the brain downward around the pharynx, to which they send off heavy nerves (*p.n.*) on either side, the circum-

esophageal commissures (*c.c.*) merge into the sub-esophageal ganglion (*s.g.*). (The section drawn is slightly anterior to the point of union of the commissures.) The pharynx wall consists of an epithelium (*p.e.*) surrounded by a thin layer of muscle and connective tissue (*n.p.*). The epithelium itself (Fig. 2) is composed of columnar cells with oval nuclei, most of which contain a prominent nucleolus. They are, indeed, very similar to the cells of the epidermis, perhaps being a trifle smaller. A point to be particularly emphasized is that *the nervous tissue of the brain is bounded on the side toward the pharynx by enveloping layers of non-nervous tissue, that the pharynx epithelium is bounded on the side toward the brain by layers of non-nervous tissue, and between the two organs is some free space.* Nowhere is nerve tissue in intimate association with the pharynx except where the lateral nerves join it.

Within a few hours after the operation the wound closes over, and only a scar marks the region in which it was made. Cross-sections of an early stage of regeneration, one week after the operation (Fig. 3), show the epidermis entirely healed, but the region of the wound (*w.*) is readily distinguishable through the absence of the characteristic gland cells. Below the epidermis, extending down through the cut muscle layers, across the cœlomic space and joining the pharynx wall, is a dense mass of deeply stained cells (*r.t.*). Fibers extending from the cut end of one commissure pass through this cell mass just above the pharynx to the cut end of the other commissure. There is little doubt that these are nerve fibers and that they are outgrowths of cells of the old nerve tissue. Lying approximately mid-dorsally and exactly in the pathway of these fibers is a small and not definitely limited group of cells (*br.f.*) made conspicuous by their especially intensive staining. These cells, containing large nuclei with prominent nucleoli, are similar to the cells of both pharyngeal and epidermal epithelia. Their position, corresponding precisely to that occupied by the obvious brain fundament of later stages, as well as their character, marks them as the early brain fundament. (The sections in this series were not exactly transverse. The section represented in Fig. 3 contains a slightly lateral portion of the brain fundament.) There is no line of demarcation between the brain

fundament and the pharyngeal epithelium. One merges uninterruptedly into the other.

Fig. 4 is a high-power drawing of that region of the pharyngeal epithelium marked *X* in Fig. 3, together with the neighboring portion of the brain fundament. The connective tissue and muscle which in the normal pharynx lie external to the epithelium (Figs. 1 and 2, *n.p.*) have entirely disappeared from this region of the pharynx wall. Not only has the dorsal pharynx wall lost its external layer, but its epithelium shows signs of considerable activity. Instead of being composed of tall columnar cells with fairly well-defined walls, the epithelium has become syncytial. Its nuclei are larger than the normal epithelial nuclei and more nearly spherical, but, like the normal, show the usual prominent nucleolus. Two mitoses appear in the part of the section drawn (Fig. 4, *m.*) and numerous nuclei of the epithelial type lie in more or less deep positions. The whole appearance of this syncytial cell mass is indicative of rapid cell proliferation.

In sharp contrast with these conditions are those found between the brain fundament and epidermis (see Fig. 3). The distance between the brain fundament and the epidermis is very much greater than that between the brain fundament and pharynx epithelium. The mass of cells between brain fundament and epidermis consists mainly of cells with small deeply stained nuclei without prominent nucleoli, resembling therefore neither the epidermal nuclei nor those of the brain fundament. They remind one rather of leucocytes. Here and there within this mass is found an occasional nucleus which is similar to those of the epidermis. No mitosis could be observed in the epidermis. The presence of mitosis at this stage in the pharynx epithelium and its absence in the epidermis is significant.

At a twelve-day stage of a case in which the wound was made dorso-laterally, the brain fundament approaches normal form, consisting of two enlargements connected mid-dorsally. The position of the wound is readily distinguishable by the absence of differentiated epidermal gland cells. The cut through the muscle layers is now completely healed, but its location is marked by the presence of more than the usual number of nuclei. There is no mass of wound tissue extending from the healed epidermis down to the

pharynx as there was in the seven-day stage. Some of the nuclei in the region where the muscle layers have been repaired resemble epidermal nuclei, but no mitosis can be observed in the epidermis. Lying about midway between the epidermis and pharynx epithelium is the brain fundament. Fig. 5 represents the median region of the brain fundament and the neighboring pharynx epithelium. Between the brain fundament (*br.f.*) and the pharynx epithelium (*p.e.*) occurs a compact cell mass similar to that found in a corresponding place in the seven-day stage. The dorsal pharynx epithelium in this worm is still active. The non-epithelial tissue of the pharynx wall terminates on either side, leaving the mid-dorsal region of the epithelium in close relation to the new brain. Throughout this series of sections mitoses (*m.*) are fairly abundant in that part of the dorsal epithelium lying beneath the brain fundament, but not elsewhere. The occurrence of these mitoses, together with the presence of nuclei precisely like those of the epithelial layer, but sub-epithelial in position (Fig. 5, *s.n.*), affords the best possible evidence that the pharynx epithelium is proliferating cells which are passing into the region of the regenerating brain. Smaller nuclei, probably leucocytic in nature, are found interspersed with these other nuclei. Some fibrous material (*f.*), probably indicating the reëstablishment of the muscle and connective tissue layer of the pharynx wall, and also blood vessels (*bl.v.*), are observed between the pharynx epithelium and the brain.

In a nineteen-day stage the new brain has a normal shape, but is not yet of normal size. Very little evidence of the wound remains aside from the absence of differentiated gland cells in the mid-dorsal epidermis. The wound in the muscle layer is barely recognizable. Between the brain fundament and the pharynx epithelium lies a compact cell mass as in earlier stages, but with some indications of the establishment of the non-epithelial tissue of the pharynx wall. Numerous cases of mitosis occur in the dorsal pharyngeal epithelium. Several cases of mitosis within the brain fundament demonstrate that the cells already there are increasing in number.

In order to put the epidermis at the greatest possible disadvantage as a source of material for the dorsal brain anlage, a number of operations were performed in which the incisions were

made either laterally or ventro-laterally. If, now, the epidermis is to provide cells for the regenerating brain, such cells must arise from the uninjured mid-dorsal epidermis and pass through the muscle layers to the region where the brain should develop, or else they must migrate there from the lateral epidermal wound. Either because of the severity of this operation or because of the weakened condition of worms kept through the winter, very few of these worms lived. One of the survivors was fixed after ten days' regeneration. In sections (Fig. 6) through the brain region of this worm the lateral location of the wound (*w.*) is recognizable by a break in the muscle wall and the lack of differentiated gland cells in the overlying epidermis. The wound is remote from the brain fundament (*br.f.*) which is already clearly established in its normal mid-dorsal position. While the break in the body wall is filled with regeneration tissue (*r.t.*), this tissue does not extend across the cephalic space to the brain fundament. In this operation the lateral nerves were left intact and there is no evidence that the pharynx epithelium was injured at any place. Nevertheless, as regards relation of brain fundament to the pharynx wall, we find conditions here the same as in cases described above—an undoubted activity on the part of the dorsal pharynx wall, as evidenced by its lack of the external connective tissue and muscular layer and by continuity of the proliferating epithelium (*p.e.*) with the regenerating brain (*br.f.*). The brain anlage appears as a mass of deeply stained cells lying mid-dorsally in the course of a tract of fibers which have grown across between the two commissures. Most of these cells are of the characteristic nervous and epithelial type. Interspersed with these are smaller nuclei of doubtful significance, probably leucocytes and connective tissue elements. Since in this case the epidermis as a source of new brain material is eliminated, there remain two possible sources—the pharynx epithelium and the old nervous tissue. However much the latter may supply to the new brain fundament, it is certain that the pharynx epithelium plays an important part.

While the evidence in a problem of this sort is necessarily indirect, since one can not watch the process of regeneration within the living worm, but is compelled to rely on the study of fixed and

sectioned material, nevertheless, in the twenty-five worms which were carefully studied, the following facts stand out significantly:

1. While there is evidence in some cases that the epidermis is contributing cells to the regenerating brain, there is much more evidence of the same kind that the pharynx epithelium is contributing.

2. In nearly all cases the brain fundament is in closer relation to the pharynx epithelium than to the body wall.

3. An uninjured dorsal epidermis does not become active and contribute to the new brain, while an uninjured dorsal pharynx epithelium does.

4. When the incision is made decidedly laterally, there is no evidence of migration of cells from the wounded epidermis to the new brain.

5. It is certain that the old nerve cords contribute some material to the regenerated brain. Just how much it is impossible to say.

6. The cells which seem to be passing from the pharynx epithelium into the brain fundament have nuclei precisely like those of cells which, in the later stages of regeneration, are certainly to be identified as neuroblasts.

If, as all the evidence described above clearly indicates, the pharynx epithelium of the earthworm plays a large part in the regeneration of a central nervous organ, we have here the phenomenon of a tissue specialized for one purpose giving rise to one highly specialized for another and very different purpose. Still more remarkable is the fact that the pharynx, which in ontogeny has nothing to do with the origin of the nervous organs, should, upon removal of the brain, become an important source of material for its regeneration. The only morphogenetic relation between the old nerve tissue and the pharynx epithelium is that both are ectodermal in origin, since the latter develops by invagination of ectoderm.

This case resembles very closely the regeneration of the salamander's lens as demonstrated by Wolff's (1894, 1895) experiments on Triton. The lens, which in ontogeny is formed by a thickening and invagination of ectoderm, is regenerated from the upper edge of the iris. It can hardly be imagined that, in nature, an earthworm accidentally loses a brain or the salamander a lens.

Therefore the tissues concerned in regenerating these structures could hardly have acquired that power through natural selection. In both cases the source of the regenerating material is an epithelium which, originally ectodermal, has come to constitute a specialized part of an internal organ and yet has not lost its primitive ectodermal potentialities, or "totipotence."

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ABBREVIATIONS.

bl.v......Blood vessel.
br.....Cephalic ganglia ("brain").
br.f......Brain fundament.
c.c......Circum-esophageal commissure.
c.m......Circular muscle layer.
c.t......Connective tissue.
c.t.s......Connective tissue sheath.
e.....Epidermis.
f.....Fibers.
l.m......Longitudinal muscle layer.
m.....Mitosis.
n.p......Non-epithelial (muscular and connective tissue) layer of pharynx wall.
p.e......Pharynx epithelium.
p.n......Pharyngeal nerve.
r.t......Regeneration tissue.
s.g......Sub-esophageal ganglion.
s.n......Sub-epithelial nuclei.
w.....Region of wound in epidermis.

EXPLANATION OF FIGURES.

1. Diagrammatic cross-section through the brain region of a normal earthworm. × 50.

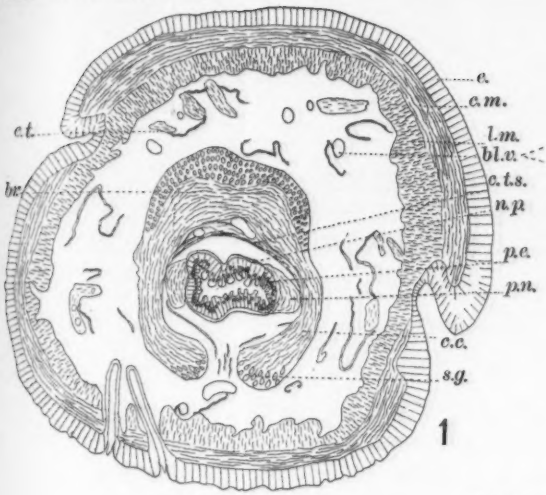
2. Section of mid-dorsal pharynx wall in the brain region of a normal worm. × 250.

3. Diagrammatic cross-section through the region of the regenerating brain 7 days after the operation. The section drawn was not precisely transverse and shows a slightly lateral portion of the brain fundament. × 50.

4. High-power drawing of that portion of Fig. 3 indicated by *X*. × 300.

5. Section through the mid-brain region of a worm at the 12-day stage of regeneration, together with the pharynx epithelium lying immediately below that region. × 300.

6. Diagrammatic cross-section through a worm at the 10-day stage of regeneration. The incision was made in a lateral position (*w*.). × 40.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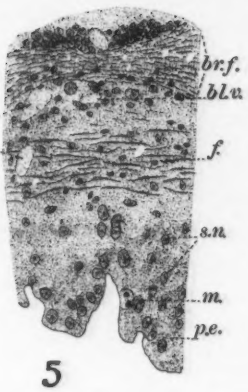
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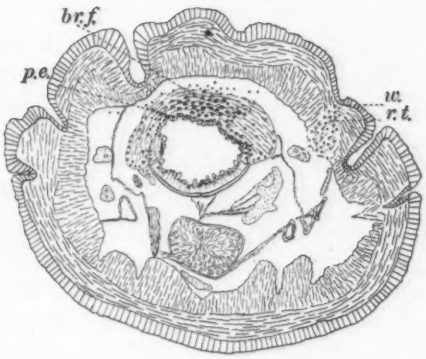
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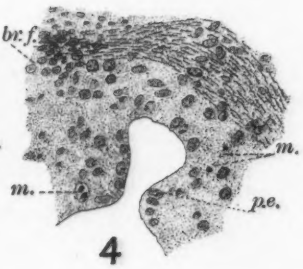
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BOTRYLLUS SCHLOSSERI (PALLAS): THE BEHAVIOR OF THE LARVA WITH SPECIAL REFERENCE TO THE HABITAT.[1]

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UNIVERSITY OF MAINE.

The purpose of the work reported in this paper was to determine more accurately than had been possible previously, the nature and effect of certain of the responses of the larva of *Botryllus*, and to investigate more fully the relation they bear to the apparent "selection" on the part of the larva of the place for attachment preparatory to metamorphosis. The work was done during the summer of 1923, at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Thanks are due to the university of Maine for making the work possible, to the Marine Biological Laboratory for the facilities for the work, and in large measure to Dr. Caswell Grave for his helpful suggestions and generous assistance.

During the course of previous experiments, some evidence appeared indicating that the *Botryllus* larva tends to "select" definite places of attachment for metamorphosis. Larvæ were allowed to undergo metamorphosis in crystallization dishes containing blades of eel grass stretched obliquely from the bottom of one side of the dish to the top of the other, and held in place by glass slides. When sufficient time had elapsed for metamorphosis to take place, the location of the zooids was noted. Measurements of the eel grass, the diameter of the dishes, and the depth of water were made in each case, and calculations were made to determine the total area available to the larvæ for metamorphosis, and the percent of that area which was offered by the eel grass. Similarly, the percentage of larvæ metamorphosing on the eel grass was

[1] This paper is supplementary to one previously written by Dr. Grave and myself on the same species of *Botryllus*. It may be found in the *Journal of Morphology*, Volume 39, Number 1.

determined. The results of six such experiments—including the four previously reported—are indicated in the following table.

TABLE SHOWING THE RESULTS OF SIX EXPERIMENTS DESIGNED TO DETERMINE WHETHER OF NOT THE LARVÆ "SELECT" EEL GRASS AS A PLACE FOR METAMORPHOSIS.

Experiment.	Per Cent. of Metamorphosing Zooids Attached to Eel Grass.	Per Cent. of Total Area Offered by the Eel Grass.
1	55.8	11.1
2	48.1	18.3
3	55.2	20.0
4	43.7	19.4
5	12.0	14.4
6	32.8	17.6
Average	41.23	16.8

These results indicate that the larvæ under these laboratory conditions, tend to "select" eel grass as a place for permanent attachment and metamorphosis. It seems safe to assume that the responses of the larva which tend to bring it into contact with eel grass under the conditions in the experiment, would function in a similar way in the natural habitat of the larva. Granted, then, that a certain amount of "selection" does take place in determining the place of metamorphosis, what are the specific responses of the larvæ—and what the stimuli bringing about these responses—whereby the larva is led to a place suitable for metamorphosis? Does the eel grass offer any chemical attraction for the larvæ? Does the larva show a positive response to gravity during the latter part of its free-swimming period, thus in its natural habitat coming in contact with the lower parts of the eel grass blades? Is the negative response to light responsible for this "selection"? Does the larva tend to follow surfaces, thus keeping it in a favorable locality once it has reached it? Does the response of the larva in swimming upward when renewing activity play any part in determining its place of attachment and metamorphosis? These are some of the questions which suggested themselves. Enough of them have been answered to suggest at least which factors function in determining the place of metamorphosis of the larvæ.

Chemical Attraction.—In considering the question of the "selection" of the eel grass by the larvæ, it seemed at first quite pos-

sible that the eel grass might give off some substance to which the *Botryllus* larva orients. The result of one experiment, however, indicated plainly that this was not the case. Clean blades of eel grass were stretched between the ends of a piece of coarse wire netting, bent into the shape of a flat U. The blades were so arranged that their flat surfaces were parallel to the central portion of the wire netting. This apparatus was suspended near the bottom of a large aquarium at the least illuminated side. The only source of light was an opening in the covering of a north window of approximately the same size as the aquarium. Since the aquarium stood immediately in front of this opening, the intensity of the light on the two sides of the eel grass blades was practically the same, and the possibility of light intensity playing any part in determining the location of the larvæ on the eel grass was thus largely eliminated. Larvæ were introduced into the aquarium immediately after liberation from the parent colony, and were allowed to metamorphose there. When sufficient time had elapsed for attachment to have taken place, records were made of the location of the zooids. It was expected that if the eel grass gives off a chemical substance to which the larva orients, at the time when metamorphic changes are about to begin, the metamorphosing zooids would be found on the eel grass. Although larvæ had been observed to swim about the grass blades, not one metamorphosing zooid of the several hundred in the aquarium, was found attached to them. This result indicates plainly that chemical attraction is not a factor in determining the place of attachment of the larvæ for metamorphosis.

Response to Gravity and Light at the End of the Free-swimming Period.—In the experiments first described in which larvæ were allowed to metamorphose in crystallization dishes containing blades of eel grass stretched from the bottom of one side to the top of the other the factor of gravity would have but a small part in determining the place of metamorphosis of the zooid because of the shallowness of the water. It seemed as if in its natural habitat, a positive response of the larva to gravity at the time of metamorphosis would be advantageous, since it would tend to bring the larva into contact with a place suitable for metamorphosis, such as the lower portion of eel grass blades. Previous

work, however, had shown that although on casual observation larvæ appeared positive to gravity at the end of the free-swimming period, the response of the larva was not as simple as at first considered. After experiments of many types had been performed, the conclusion was reached that at the end of the free-swimming period, larvæ are indifferent in their response to the stimulus of gravity. One experiment is sufficient to illustrate. Larvæ just liberated from the parent colony were allowed to metamorphose in a cylinder of 500 c. c. capacity held in an oblique position at an angle of about 45 degrees. The effect of light was eliminated in one case by jacketing the cylinder completely with black paper; in the other three, by performing the experiments in a dark-room. After the time necessary for metamorphosis had elapsed the cylinders were examined. The attached zooids were found evenly distributed from the top to the bottom on the lower side of the cylinder in three of the four cases; in the fourth, zooids were much more frequent near the top of the cylinder than the bottom, but as in the other three cases, they were much more frequent on the lower than the upper side. If the larvæ are positive in their response to gravity at the end of the free-swimming period, it was to be expected that the metamorphosing zooids would be found attached to the lower end of the cylinder. The fact that larvæ were not found in large proportion at the bottom of the cylinders, shows clearly that they are not positive in their response to gravity at the end of the free-swimming period. Their even distribution would indicate that the larvæ pass thru a long period of indifference to gravity during which, in the absence of light, random movements send them to all parts of the container, and that when metamorphic change sets in, the larvæ become inactive, sink, and attach themselves to the first surface with which they come in contact.

The question which naturally follows, is why, in the absence of a positive response to gravity, the larvæ metamorphose at the bottom of a cylinder held in a vertical position, as has been shown to be the case in a former paper. There are two possible explanations. If the larvæ attach and begin metamorphosis on the surface with which they first come in contact when swimming movements cease, as appears to be the case when confined

in a glass cylinder, the bottom of the container must of necessity become the place of attachment of a large proportion of the zooids present in it. The remainder which metamorphose elsewhere get caught at the top of the water or near small bubbles of air on the side of the cylinder by surface tension, and still others, in sinking strike the sides of the container, and attach before reaching the bottom. A second explanation for the occurrence of metamorphosis at the bottom of a container standing in a vertical position, may be found in the fact that the larvæ are negative in their response to light at the close of the free-swimming period. Two experiments will serve to illustrate. Larvæ just liberated from the parent colony were allowed to metamorphose in a cylindrical graduate placed vertically, the sides of which had been partially covered with black paper. Three bands of black paper covered the areas between the 100 and 200 cc. marks, the 300 and 400 cc. marks, and an equal area above the 500 cc. mark. Thus alternating bands of light and shadow, having the same areas were produced. Of the 127 larvæ which attached to the cylinder, 82 attached in the darkened areas, five to the sides in the undarkened areas, and forty on the bottom. At another time larvæ just liberated from the parent colony were introduced into a glass cylinder of 1000 cc. capacity the sides and top of which were completely jacketed with black paper. The cylinder was held in an oblique position at an angle of less than 45 degrees, and a 75-watt nitrogen bulb was so placed that rays of light parallel with the long axis of the cylinder passed into the column of water thru the uncovered bottom. When sufficient time for metamorphosis had passed, the location of the metamorphosing zooids was noted. A very large proportion were found at the upper end of the cylinder, away from the source of light. These results indicate that the larva is not positive in its response to gravity at the end of the free-swimming period, but that its negative response to light is effective in leading it to the darkest part of the container. The lowest part of the container, and in its natural habitat, the lower levels of water are in general the darkest regions to which the larva can gain access. Hence the negative response to light performs very much the same function as would a positive response to gravity.

Other Responses.—In addition to these responses of a type commonly studied, the larvæ showed a reaction of another sort which appears to be of significance in relation to its habitat. One phase of this reaction, the response of turning abruptly upward when stimulated by a passing shadow, has been mentioned in the paper by Dr. Grave and myself. It was found on further observation that this abrupt upward turn occurred not only when the larva was stimulated by a passing shadow, but that it is a common response at other times also. As has been previously noted, the larva does not swim continually, but passes thru alternating periods of activity when it swims vigorously, and inactivity, during which it sinks, motionless thru the water. The behavior of one hundred larvæ on resuming activity after a period of quiescense was noted and recorded. Nineteen of the larvæ on resuming activity swam downward, seventeen swam in a horizontal direction, and sixty-four swam upward. These and other similar observations indicate that the larva has a strong tendency to swim upward when stimulated to renewed activity after a period of rest. What determines this response is a separate question. One stimulus, quite evidently, comes from the passing of a shadow; others, in all probability, are internal in origin.

It was noted also that larvæ frequently swim upward when they come in contact with a vertical surface. The behavior of one hundred larvæ on coming in contact with the vertical wall of the container was noted. Of these, sixteen attached themselves temporarily to the container at the point of contact; sixteen swam downward; seven swam in a horizontal direction, and sixty-one swam upward. At another time similar observations were made on the behavior of larvæ on coming in contact with a piece of black paper suspended in a perpendicular position in the aquarium. Of the hundred cases observed, two ceased activity and sank; eleven swam along the surface in a horizontal direction, and eighty-seven in an upward direction. These results suggest that the larvæ have a tendency to follow surfaces with which they come in contact in an upward direction, particularly if the surface is opaque.

Do these various reactions—the responses to light and gravity, and the "habit" of swimming upward when resuming activity or when coming in contact with a perpendicular surface—have an adaptive value to the species? When considered in relation to the natural habitat, they assume considerable significance. Adult colonies of *Botryllus* are attached very commonly to eel grass, or other objects projecting above the bottom, such as the submerged parts of floats, or rockweed. They are always found below low tide mark, and never on the bottom, being found only on the parts of eel grass blades which are not exposed at low tide, and the submerged parts of logs. Sand or mud on which the eel grass thrives offers no place for attachment and metamorphosis to the larva. It is evident from a study of the distribution of the adults that those larvæ which attach too near the surface or which sink to the bottom do not reach the adult condition. The responses of the larva are such as to keep it away from these unfavorable environments. The positive response to light and the negative response to gravity at the beginning of the free-swimming period serve to bring the larva to the surface and to distribute it more widely than would be probable without these responses. There follows a period of indifference when the larvæ show random movements; they do not orient to stimuli either of light or gravity. During this period the adaptive value of the response of swimming upward when resuming activity, or when coming in contact with a surface is evident. The negative response to light, were it unmodified by these additional responses might force the larva at the time of metamorphosis into a position too near the bottom. The response of swimming upward keeps it away from the bottom, and tends also to keep the larva in proximity to objects suitable for attachment. In experiments such as were first described, in which larvæ were allowed to metamorphose in crystallization dishes containing eel grass blades stretched obliquely from the bottom of one side to the top of the other, larvæ were many times observed to be trapped by these responses in the region of the grass blades. Larvæ sinking motionless thru the water, frequently came within range of shadows cast by the grass. Instantly they would resume activity in an upward direction.

Sometimes this resulted in bringing the larva in contact with the eel grass immediately, in which case it would follow up the surface of the blade for a few centimenters before lapsing again into inactivity. More often it failed to touch an eel grass blade, and after swimming upward a centimeter or so, it would relapse into inactivity, only to be stimulated again as it sank into the shadow a second time. The value of these responses to the larva when in its natural environment is strikingly evident.

If, as it appears, these responses function in a way which tends to lead the larva to a suitable place for metamorphosis, we have a basis for the belief that they are of survival value to the species, and that they have been in the past and perhaps are still operative in its continued evolution.

THE CHEMICAL SENSE AND FEEDING BEHAVIOR OF *NEREIS VIRENS*. SARS.

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In 1873 Verrill stated in his report on the invertebrates of Vineyard Sound that the clam worm, *Nereis virens*, "feeds on other worms and various kinds of marine animals. It captures its prey by suddenly thrusting out its proboscis and seizing hold with the two terminal jaws; then withdrawing the proboscis, the food is torn and masticated at leisure. . . ." Maxwell (1897) confirmed Verrill's conclusions as to the character of its food. He found that if a small piece of worm on the end of a needle is placed within reach of a normal *Nereis*, the animal seizes the food and devours it. More recently, however, Gross (1921) has failed to find any evidence that *Nereis virens* is a carnivorous worm; concluding that it feeds principally upon plant life. This verdict was based upon studies in the laboratory and in the field.

Our own observations began with dropping crushed periwinkles [*Litorina littorea* (Linn.)] in shallow water at low tide in order to study the responses of crustaceans. That such bait would cause *Nereis* to react was a thought that had not occurred to us, so that our surprise can easily be imagined when presently a worm extended the anterior end of its body from below smooth sand, moved toward the snail, seized and quickly jerked it down into its burrow. What we saw was a confirmation of Verrill's statement noted above.

Subsequent tests with the same food resulted in numerous responses of a similar nature until it appeared that *Nereis* was reacting with remarkable precision to small amounts of materials emanating from the crushed snails. A worm usually emerged several centimeters from the snail, and advanced rather slowly over the surface of the sand by movements of the body and parapodia toward the bait, which it finally seized in its powerful jaws and drew rapidly to its burrow by a sudden

muscular contraction. *Nereis* responded in the same way to all other forms of animal food which were offered, viz., crushed clam [*Mya arenaria* Linn.], mussel [*Mytilus edulis* Linn.], bits of fish [*Fundulus heteroclitus* (Linn.)], and meat of the blue crab [*Callinectes sapidus* Rathbun]. When the food was grasped it was always pulled to the mouth of the burrow and, unless too large, into the interior of the burrow itself. In no case was a worm observed to leave the burrow entirely and move about freely in the water.

In order to learn something of the extent to which the animals depend upon a chemical sense in such reactions a number of tests were made, one of which was carried out in the following manner. The position of a worm was first determined by baiting with a piece of clam which was removed before the animal had a chance to seize it. Some fragments of the same food were then wrapped in cheese cloth and dropped into the water about five centimeters from the opening of the burrow. Equidistant from the burrow and one centimeter from the first packet was placed a second, consisting of a white pebble done up in cheese cloth. Each packet measured slightly over two centimeters in diameter and both had essentially the same appearance. The worm soon responded by emerging from the burrow and, advancing in a straight line toward the *baited* packet, seized it and dragged it down almost out of sight. The packet was then dug out and replaced in its former position. In a few minutes the worm reappeared and repeated the reaction in every detail. Again the food was recovered, and this time the positions of the baited and unbaited packets were interchanged. Within several minutes the anterior end of the worm came out of the sand somewhat nearer to the packets than before and moved forward in a course which, if adhered to, would have brought it between them. However, when close to the packets the animal suddenly turned and fastened its jaws in the baited one and for the third time pulled it into the sand.

The results of this experiment indicate that sight plays little or no part in the worm's food reactions under the conditions prevailing. Tests were also made by dropping pebbles near occupied burrows to ascertain if agitation of the water, or some

physical factor, influenced the animal's response. To such stimuli no response occurred, although the worms appeared promptly when crab's meat was placed in the same situations. The evidence, therefore, supports the view that a chemical sense is the primary one upon which its responses to animal food depend.

Certain striking features of recorded behavior may now be considered. It was early noted that when a worm appeared from beneath the sand in response to chemical stimulation there was little uncertainty shown in the direction of its movements. Almost invariably it advanced toward the bait which, if not too far away, was generally found without difficulty. It was also observed that, when the food was out of reach, the worm withdrew into its burrow only to reappear, frequently in a position nearer the source of the stimulating material. In order to study these directive reactions further a number of animals were collected and brought into the laboratory. They were kept for a time in dishes containing water and sea lettuce, and under these conditions they would often take from forceps small pieces of meat offered them. In fact, all of the foods to which the worms reacted in their natural habitat were also accepted as they moved about in the folds of sea lettuce, enclosed more or less by mucous secretions. Under these conditions, however, they were easily disturbed and their somewhat erratic feeding behavior indicated that they were unfavorably situated for any detailed experimental study of their chemical reactions. Accordingly four worms were placed in a circular glass dish having an inside diameter of 28 cm. and containing sea water and sand. The layer of sand was approximately 2.5 cm. deep. The animals immediately entered the sand, forming burrows lined with mucus which here and there connected with the surface by well marked openings. They showed no tendency to leave the burrows if the dish remained undisturbed. After preliminary tests, which demonstrated that the worms would react to food juices as readily as they did in their natural surroundings, three similar experiments were performed, one of which is described in some detail in the following paragraphs.

August 3, 1923.

9:32:00 A. M. The water was gently stirred with a glass rod and the dish kept under observation for ten minutes. The worms remained in the sand.

9:51:00. A clam (5.4 cm. in length) cut up on half shell was placed in the center of the dish and the water stirred as before. The worms appeared as follows: worm I at 9:52:15, seven centimeters from the edge of the dish; II at 9:53:17; III at 9:54:45; IV at 9:56:50. Worms II, III, and IV appeared at the periphery of the dish.

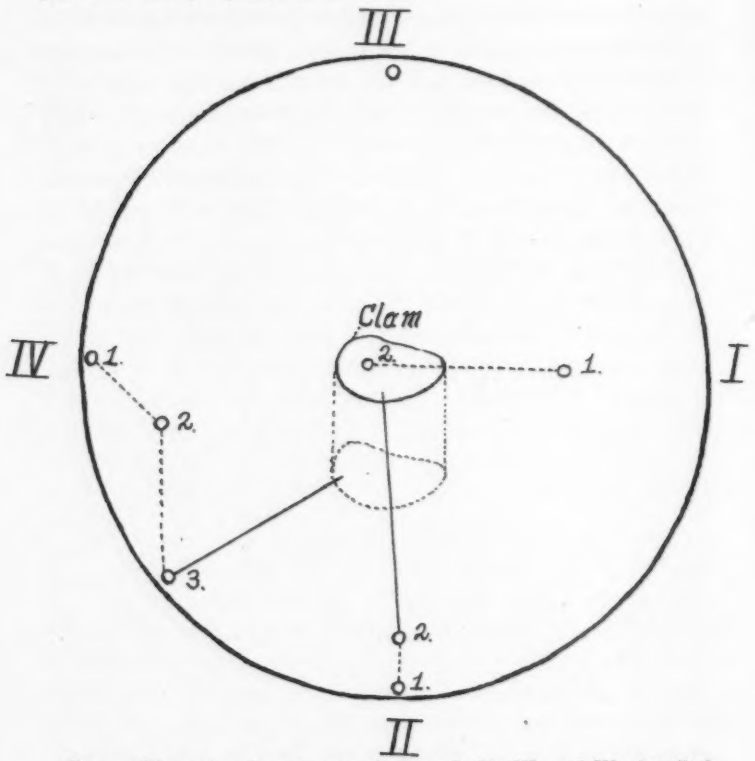

FIG. 1. Illustrating the responses of worms I., II., III., and IV., described in the text. The arabic numerals denote successive appearances of individual worms.

Worm I.—At its first appearance it made a few wavering movements with the anterior segments of its body at the mouth

of the burrow and then withdrew. The same reaction was repeated a few minutes later. Finally at 10:07:30 it was found with its head exposed directly beneath the clam to which point it had burrowed, a distance of 7 cm.

Worm II.—It repeated its first appearance at the edge of the dish, withdrawing quickly each time. At its third appearance the worm proceeded directly toward the clam, exposing about 10 cm. of its body, and then quickly disappeared within its burrow. At 9:59 it came out of the sand 2 cm. nearer the clam, stretched out until it reached the clam, but finding a small fragment nearby, it seized the fragment and withdrew without disturbing the clam. At 10:07:30 the worm again emerged, this time within 2 cm. of the clam, which it grasped and dragged a distance of 3 cm., partially burying its prize in the sand. When the clam was dislodged the head of worm I. was disclosed directly beneath. At 10:13:30 worm II. reappeared at the mouth of its original burrow at the edge of the dish but made no effort to reach the clam.

Worm III.—Emerging first at the edge of the dish it immediately moved toward the clam, not quite reaching it. After withdrawing it came out again at the same point at 10:11, but by this time the clam had been pulled 3 cm. away by worm II. It drew back into its burrow and was not seen again.

Worm IV.—When first noted at the edge of the dish the worm made a few wavering movements and then disappeared, coming up again for a short time 3 cm. away. Finally at 10:17:30 it was observed at the edge of the dish, but 10 cm. from the first opening. It advanced directly toward the clam, seized it but failed to move it because of the position in which it had been wedged in the sand by worm II. After tugging at it a while, and probably biting off a piece, the animal withdrew.

The results of this experiment and two others carried out in the same way confirmed beyond question our observations made in the field. Although a worm on coming to the mouth of its burrow usually advanced the anterior end of its body in a direction toward the clam, it perhaps showed somewhat less certainty in this phase of its response than when in its natural environ-

ment. This, however, was to be expected, for in the dish well defined water currents bearing food juices, which undoubtedly aid under natural conditions in directing the animal toward the food, were largely absent, and the stimulating material must have become rather generally distributed through the water. The animals, nevertheless, after stimulation, instead of confining their activities to the periphery of the dish, near which they usually appeared, exhibited a marked tendency on failing to reach the clam to move toward the center of the dish. The explanation of how the worm maintains its direction toward the source of stimulation after withdrawing into the sand is not clear. It appears likely, however, that this directive response is a movement toward areas where the chemical substances given off by the food, and infiltrated through the sand, show higher concentration. The sense of sight plays an insignificant if any part in these reactions, for if a pipetteful of filtered clam extract is substituted for the clam in an experiment like that just described, the worms show the same responses, extending their bodies toward the center of the dish where the extract was placed.

In the laboratory as well as in natural surroundings we never saw the animals lose contact with their burrows during their movements over the sand in response to food excitation. They were tested by moonlight in the laboratory with food but no difference from behavior in daylight was noted.

For some time after the first observations on *Nereis* were made we were unable to explain how the stimulating material derived from the small quantity of food used in some of our experiments penetrated the burrows in sufficient amounts, and quickly enough, to call forth such prompt reactions. The solution of this problem was found when worms were allowed to enter glass tubes, open at both ends, the calibers of which were nearly the same as those of their burrows. Tubes of this sort are soon lined with mucus and the worms remain in them for hours at a time, exactly as they do in the sand. No difficulty was experienced in inducing them to enter the tubes. It was only necessary to direct the head into the opening, after which the worm moved forward of its own accord; and once well inside stopped locomotion. In the tube *Nereis* exhibits a most striking

form of behavior which may be described as a rhythmic undulatory movement of a portion of the body taking place in a dorso-ventral direction, the parapodia remaining passive. These muscular waves were sometimes limited to the anterior end, in which case a nodding of the head occurs; at other times they appeared only at the posterior end, or perhaps in a position nearer the middle of the body. The movement is not uninterrupted but never discontinued for any length of time. This activity produces a current in the water which passes along the animal's body from the anterior to the posterior end.

That these body movements also occur when the animals are in their burrows was clearly demonstrated by two individuals kept in glass dishes containing sand. In one instance the nodding of the head mentioned above was seen through the glass as the animal lay in its burrow at the side of the dish. Carmine grains dropped in the water a few centimeters above an opening of the burrow were immediately drawn inside. In another case a long burrow was formed against the glass and here the undulatory movements of the worm were distinctly visible. From this burrow three tunnels led to openings at the surface close to the glass. From this it is clear how *Nereis,* concealed in its passageways within the sand, receives not only a constant supply of fresh water but also may be stimulated by any chemical change in the water above.

Worms occupying glass tubes respond to chemical stimulation of food as readily as those in burrows. Sea water squirted from a pipette close to a tube results in no response, but when a filtered extract of clam is introduced in the same manner the worm starts forward, moving quickly toward the end of the tube toward the juice, thrusting out its head and seizing a bit of clam dropped near the tube, or held in the forceps. This reaction was observed many times and with several individuals, and appears to be subject to remarkably little variation. By use of the glass tube every movement of the worm can be seen perfectly, and the method promises to be an excellent one for the study of the distribution of the receptors involved in the responses. Experiments designed to throw light on this aspect of the problem are already under way and will be reported upon at a later time.

SUMMARY.

1. *Nereis virens* is carnivorous, although in the absence of other food it has been observed to feed upon sea lettuce. Under natural conditions it undoubtedly is omnivorous, since Gross has found evidence of the presence of plant food in the digestive tract.

2. *Nereis* was never observed to leave its burrow when baited with meat of various marine animals, but it may expose all except the posterior segments of its body in reacting to the bait. This does not mean that the worm may not leave its burrow under other circumstances. The animal is highly thigmotactic.

3. There is positive evidence that *Nereis* depends upon a chemical sense in finding animal food; sight playing little if any part in the act. Currents in the burrow produced by an undulatory body movement are undoubtedly a factor in conveying food stimuli to the sense organs.

4. *Nereis* shows a marked tendency to extend its body from the burrow in the direction of food, and failing to reach it, to reappear in a new position nearer the source of the stimulating material.

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NEMATOCYSTS OF *MICROSTOMA*.

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For a long time the nematocysts of *Microstoma* were considered to be structures that the worm elaborated as did *Hydra*. Martin ('08) published an account of the manner in which *Hydra* is attacked by *Microstoma* and indicated his conviction that the namatocysts of *Microstoma* were derived from the Hydras that had been eaten. Kepner (11) published an account of the histological details involved in the process of transporting the nematocysts of *Hydra* from the enteron to the epidermis of *Microstoma*. In this account the inference was made that this complicated series of phenomena must mean that *Microstoma* handled these nematocysts in this manner in order to use them. This inference was early challenged by Glaser (11). A tentative reply was given Glaser's note in *Science*, Volume 34, page 213, 1911.

The details of the processes involved when *Microstoma* deals with the nematocysts are so variable and so intricate that it was felt that these processes could mean only one of two things, namely, either this was a method of eliminating indigestible foreign bodies or a method of securing weapons of defense or perhaps offense.

From time to time since 1911 efforts have been made to determine which of the two meanings was behind this conduct of *Microstoma*. This paper represents the results of our efforts that have thus extended over a decade and have been taken up each year of this decade to a certain extent.

MATERIAL AND METHODS.

Microstoma caudatum is found rather abundantly in the early spring months in the submerged detritus along the banks of fresh water ponds about the University of Virginia. Sometimes Hydras are found living with it in great numbers; at other times

there are few Hydras. When many Hydras are present it is difficult to find Microstomas that lack nematocysts. When there are few Hydras living with *Microstoma* in nature, specimens that lack nematocysts are readily obtained.

It has been found that by placing *Microstoma* in watch-glasses containing fresh water and some fragments of water soaked, dead leaves they can be kept indefinitely; provided the air be kept free of coal gas, illuminating gases, and formaldehyde vapor.

Nematocysts Passing from Endoderm into Mesoderm.

While this paper is not dealing with the methods by which *Microstoma* handles nematocysts, it is of interest to record that Kepner and Whitlock in 1917 observed a nematocyst being carried down through the body of an endodermal cell and delivered through the basement membrane into the mesenchyme. This was a very slow process. The movement of the nematocyst could be appreciated only when its position was recorded and then five minutes later its position again observed. Its rate was not accelerated as it passed through the basement membrane into the mesoderm. The most remarkable feature of this was that the obtuse end was directed towards the path along which the nematocyst was being transported.

Nematocysts Uniformly Distributed.

Attention might be called to the additional fact that when these nematocysts are taken up by the mesenchymal cnidophages they are distributed uniformly at the epidermis by these attending cells. So that immediately after a *Microstoma* has acquired a supply of nematocysts at its surface, these nematocysts are uniformly distributed. It does not appear, however, that if the anterior end, or any other region, lose its quota of nematocysts that some will be taken from other regions of the body to take the place of those lost. In this manner specimens sometimes come to have a uniform distribution of nematocysts except for some region (e. g. "head") that is free from them. Despite this fact, the subtle manner in which the cnidophages coöperate to bring about a uniform distribution of the foreign nemato-

cysts is remarkable and lends weight to the inference that the nematocysts are being collected by the *Microstoma* to be used.

Nematocysts Retained Indefinitely.

Again if the nematocysts of *Hydra* are carried out to the surface of *Microstoma* by way of eliminating them as indigestible foreign bodies, the question arises as to why they are held so long at the surface. Within twelve hours after a *Hydra* has been ingested by *Microstoma,* the nematocysts are distributed over the rhabdocoele's body. The process of getting these objects to the surface of the flatworm is, therefore, a matter of about twelve hours. When, however, they arrive at the surface they are held there indefinitely. Even when animals are kept under adverse conditions in the laboratory and they show more and more conspicuous decline, the nematocysts are yet retained. Specimens have been seen growing weak and beginning to rupture or break up and yet retain the nematocysts over the epidermis that remained intact. This retention of the nematocysts, likewise, suggests that they have been collected for use.

An Expensive Method.

Again this is an expensive method of dealing with objects that could be thrown out at the mouth as are other indigestible materials. For in this process much energy must be spent by the endodermal cells and cnidophages in handling the nematocysts; and, further, when the nematocysts are discharged they carry with them their attending cnidophages. The cnidophages, therefore, in attending the nematocysts act against their own welfare in that in the end they lose their own lives.

Microstoma Incurs a Danger.

Even the *Microstoma* as a whole feeds upon *Hydra* at a risk. For sometimes *Hydra* turns upon *Microstoma* and eats it. *Hydra,* however, seems to have difficulty in ingesting *Microstoma* as the following observation of January 18, 1917, shows. The *Microstoma* at once played along the side of *Hydra viridis* that had been placed with it. The worm passed to and fro along the surface of the polyp's body. Twice it came to rest amidst

the bases of the tentacles. The third time it came to lie over the mouth of the *Hydra*, the latter began an effort to ingest the *Microstoma* (Fig. 1). First the *Hydra's* peristome opened up against the ventral side. This region of the peristome did not fix the *Hydra* to the *Microstoma*, for the mouth of *Hydra* glided posteriory along the ventral side of *Microstoma* and around its posterior end as indicated by the arrow in Fig. 2. When the widely expanded peristome of *Hydra* had come to be applied over a great part of the dorsal and lateral surfaces of the *Microstoma*, the *Hydra* pressed the latter between its peristomal bell and its body and appeared to have the worm in a very serious position (Fig. 3). But from this embrace the *Microstoma* soon glided. As it escaped it showed a ruptured region of its body. This wound healed in a little while and then *Microstoma* went back and played along the surfaces of *Hydra's* body. The next time it came into the tentacular zone of *Hydra*, the polyp succeeded in grasping the *Microstoma* head-on and forthwith ingested it (Fig. 4). Since, therefore, *Microstoma* incurs a greater danger in seeking out *Hydra* than it does when it feeds upon small annelids, crustacea and other small animals and plants and since, further, it does not appear to seek *Hydra* primarily for food as will be shown later, the inference is strong that it is seeking *Hydra* for some unique end.

Microstoma Able to Draw Nematocysts from its Body when Wounded by *Hydra*.

Moreover, *Microstoma* seems to have developed an adaptive secretion by its epidermal glands that may be taken to be either rhabditic glands or the homologues of such glands which are found in other rhabdocoeles. The following observations indicate the adaptive functioning of these glands. February 15, 1917, a *Microstoma* was placed with a small *Hydra* fusca. The *Microstoma* began playing about the five tentacles. Immediately the *Hydra* firmly grasped it between its tentacles (Fig. 5). The *Microstoma* escaped from the *Hydra's* embrace. As it swam clear of the polyp, two mucous masses were to be seen, one at the posterior end and one on the right side. (Fig. 6). The lateral mass grew and as it grew drew the two enclosed nemato-

cysts out from their anchorage within the worm's body and then glided along the right side of the body posteriorly to where it fused with the posterior mass, which had three nematocysts enclosed within it. This compound mass of mucus, now containing five freed nematocysts, was sloughed off from the *Microstoma's* body and left behind. After that the *Microstoma* seemed to be enveloped in a transparent mucus sheath to which small dead objects adhered and were dragged about with the *Microstoma* until they became entangled with the tentacles of the *Hydra*. These tentacles stripped the foreign bodies from the *Microstoma's* adhesive surface. Another observation of this sort was made January 29, 1917. A *Microstoma* was placed with a large budding *Hydra fusca*. It at once attacked the *Hydra*. The *Hydra* discharged a nematocyst into the anterior end of *Microstoma*. The *Microstoma* contracted so vigorously as to break apart the two nearly formed zooids that had been developing through fission. About the deeply anchored nematocyst the anterior zooid now secreted a local mass of mucus. This mass of mucus grew until it formed a column whose length was more than two-thirds that of the zooid. Next the posterior zooid encountered the bud of the *Hydra*. As it passed the *Hydra*, the latter stung it in its posterior end along the left side. About this nematocyst a local secretion of mucus appeared and as the mass of mucus grew in length the long stinging thread of the nematocyst was dragged from the *Microstoma's* body as had a mucous column dragged the nematocyst from the body of the anterior zooid. Eventually the mucous masses were discarded and the two zooids appeared to be no worse for their experience.

All the above shows an intimate relation existing between *Microstoma* and *Hydra* which is peculiar and must have some meaning while much of the above indicates that the meaning of this conduct centers about the nematocysts of *Hydra*.

Microstoma with Few Nematocysts Reacts to *Hydra* Readily.

Moreover, a *Microstoma* that has few or no nematocysts behaves differently towards *Hydra* than does one that has a com-

plete quota of nematocysts at its surface. On December 8, 1916, a *Microstoma,* that contained seven or eight nematocysts and so greatly filled with food that the pharynx of its posterior zooid was everted, was placed with a *Hydra.* Within 12 minutes the *Microstoma* had egested some of its food and had torn off a part of the *Hydra's* oral end. Two days later a second well-fed specimen, that contained but two nematocysts, was placed with a *Hydra.* This specimen ate the *Hydra* within twenty minutes. Table I., shows that of 42 specimens containing none or few nematocysts, 6 (within two minutes after being placed with Hydras) set to work trying to feed upon the polyps, but five of them got severely wounded while the sixth was eaten by the *Hydra.* Two others were eaten by the *Hydra* within twenty-four hours. Of the remaining 34 specimens, 9 had eaten Hydras in periods ranging from 2 minutes to 20 minutes; while 20 accepted Hydras in periods ranging from 24 hours to 1 hour; and 3 remained with *Hydra* either 5 or 2 days before they accepted *Hydra.* Two specimens remained with *Hydra* 24 hours and a third 4 days when they were lost or had died without having accepted *Hydra.* The conspicuous feature of Table I. is that in many cases *Hydra* was accepted within a period of minutes, many others within a period of hours, while only a few were accepted within a period of days.

Microstoma with Many Nematocysts Reacts to *Hydra* Tardily.

Table II. makes a sharp contrast with Table I. in this respect. For this table shows the reactions of 18 Microstomas, that had either many or a full quota of nematocysts. All, except specimen 15, were kept from food for a day or more and yet only one of them, specimen 7, reacted to *Hydra* within 24 hours and this one contained only about one-third of a full quota of nematocysts. Specimen 8 remained with *Hydra* 24 hours when it was lost, without having accepted *Hydra.* Except for these two specimens, all the others reacted to *Hydra* not within periods of hours or minutes but of days and that despite the fact that they had no food for at least a day. Specimens 17 and 18 were kept away from food in each case four days. They lived four

TABLE I.

NEMATOCYSTS OF *Microstoma* NONE OR FEW.

Specimen.	Number of Nematocysts.	Time with *Hydra*.	*Hydra* Eaten.	*Hydra* Not Eaten.	*Microstoma* Eaten.	*Microstoma* Stung.
11–21–16.....	None	24 hrs.	+			
12– 6–16.....	7 or 8	12 min.	+ though the specimen had much food.			
12–10–16.....	2	20 min.	+ though well fed.		−	−
12–11–16.....	6	3 min.	+	−	−	−
1–10–17.....	None	24 hrs.	+	−	−	−
1–18–17.....	2	2 min.	−	−	+	−
1–20–17.....	6–10	2 days	+	−	−	−
1–23–17.....	8	5 days	+	−	−	−
1–24–17.....	5	2 min.	−	−	−	+
1–26–17.....	1 or 2	2 min.	−	−	−	+
2–12–17.....	1	2 min.	−	−	−	+
4–19–17.....	10	2 min.	−	−	−	+[1]
7–23–17.....	Few	2 min.	−	+	−	+
7–24–17*a*.....	6	10 min.	+	−	−	−
7–24–17*b*.....	None	10 min.	+	−	−	−
8–18–17*b*.....	None	5 min.	+	−	−	−
9–18–17*d*.....	1	24 hrs.	+	−	−	−
9–18–17*e*.....	Few	24 hrs.	−	+	+	−
9–18–17*f*.....	2	24 hrs.	+	−	−	−
9–18–17*g*.....	7	24 hrs.	+	−	−	−
9–18–17*h*.....	Few	5 min.	+	−	−	−
9–19–17*a*.....	Few	24 hrs.	+	−	−	−
9–19–17*b*.....	1	24 hrs.	+	−	−	−
9–19–17*c*.....	None	24 hrs.	+	−	−	−
9–19–17*d*.....	5	24 hrs.	+	−	−	−
9–19–17*e*.....	5	24 hrs.	+	−	−	−
9–19–17*f*.....	None	1 hr.	+	−	−	−
9–19–17*g*.....	7	1 hr.	+	−	−	−
9–19–17*h*.....	None	3 min.	+	−	−	−
10– 5–17*a*.....	None	6 hrs.	+	−	−	−
10–17–17*a*.....	1	24 hrs.	+	−	−	−
10–17–17*c*.....	6–8	24 hrs.	+	−	−	−
10–17–17*e*.....	None	24 hrs.	−	+	−	−
10–17–17*f*.....	None	24 hrs.	+	−	−	−
10–17–17*h*.....	8–10	24 hrs.	+	−	−	−
10–17–17*j*.....	None	24 hrs.	−	+	−	−
10–17–17*l*.....	Few	24 hrs.	−	+	−	−
10–17–17*m*.....	Few	24 hrs.		−	+	−
9–18–17*e*.....	Few	2 days	+	−	−	−
5– 3–23.....	Few	4 days	−	+	−	−
5– 3–23.....	10	24 hrs.	+	−	−	−
5– 8–23.....	None	24 hrs.	+	−	−	−

days with *Hydra* before they died without accepting any part of *Hydra*. Specimen 16 is the most significant one of the table. It was learned by observation that *Microstoma* could live in a small vessel of spring water for about nine days without food. Many died in less time under these conditions but none passed

[1] Rather by gastric juices than by nematocysts.

TABLE II.

NEMATOCYSTS OF *Microstoma* MANY.

Specimen.	Loaded with Nematocysts.	Without Food.	With *Hydra*.	*Hydra* Eaten.	*Hydra* Not Eaten.	Nematocysts Thrown from Enteron of *Microstoma*.
(1) 9-18-17*B*	1/2	1 day	2 days	+	−	−
(2) 9-18-17*D*	1/2	"	"	−	+	Sick; see notes.
(3) 9-18-17*F*	4/5	"	"	+	−	−
(4) 9-18-17*G*	1/2	"	"	+	−	−
(5) 9-19-17*B*	1/3	"	"	+	−	−
(6) 9-19-17*C*	1/2	"	3 days	+	−	−
(7) 9-19-17*D*	1/3	"	1 day	+	−	−
(8) 9-19-17*D*	Loaded	"	"	−	+	−
(9) 9-19-17*E*	2/3	"	3 days	+	−	−
(10) 9-19-17*F*	1/4	"	"	−	+	− Sick; see notes.
(11) 9-19-17*G*	1/4	"	"	+	−	−
(12) 9-19-17*H*	Loaded	"	"	−	+	
(13) 9-19-17*H*	4/5	"	2 days	+	−	+
(14) 9-19-17*A*	1/2	"	"	+	−	−
(15) 9-19-17*A*	Loaded	No[1]	"	+	−	+
(16) 11-21-16	Loaded	9 days	"	−	+	Piece of tadpole liver
(17) 5- 6-23	1/2	4 days	4 days	−	+	accepted immediately
(18) 5-10-23	1/5	"	"	−	+	after the *Microstoma* was taken from the *Hydra* of specimen (16).

the twelfth day. With this fact in mind a "loaded" specimen was kept away from food for nine days. It was placed with *Hydra* at the end of the ninth day and remained with it for two more days without accepting *Hydra*. This was not due to the *Microstoma's* condition having been so greatly lowered that it could not accept food. For when it was taken from *Hydra* and given a piece of tadpole liver, the *Microstoma* immediately accepted it. The facts tabulated in these two tables indicate that a *Microstoma* with few or no nematocysts attacks a *Hydra* much more readily than does one with many or a full quota.

Microstoma MAY EGEST CELLS OF *Hydra* AND RETAIN NEMATOCYSTS.

Another contrast may be drawn between the conduct of a *Microstoma* that has few or no nematocysts and that of one that has a full quota of nematocysts. If a *Microstoma* that contains a *Hydra*, so long ingested that the polyp has been reduced to a pulp, be placed under slight pressure it will discharge

[1] Not starved since it had eaten *Hydia* as specimen 14.

the green protoplasmic mass of the *Hydra's* body and retain the nematocysts within its enteron. This does not appear to be due to the fact that the pressure has held fast the mass of nematocysts; for, during the process of egesting the other *Hydra* material, the nematocysts are being thrust to and fro within the enteron. Under such condition a *Microstoma,* therefore, rejects the food and retains the nematocysts.

Microstoma May Egest Nematocysts and Retain Cells of *Hydra.*

The senior author saw just the reverse of this. A *Microstoma* was loaded with nematocysts by being fed a *Hydra.* It was then starved until it would accept *Hydra.* While the *Microstoma* was kept under observation the *Hydra* was digested. When alimentation had been completed the indigestible nematocysts were thrown out of the mouth and rejected. When Dr. W. H. Taliaferro, now of Johns Hopkins University, was told this he expressed skepticism; his challenge was accepted by the senior author to have this demonstrated. From 10.58 A.M. to 11.14 A.M., September 19, 1917, Dr. Taliaferro kept the "loaded" *Microstoma* under observation. During this time he could see the nematocysts of a recently ingested *Hydra* within the enteron. At 11.14 A. M., he saw the nematocysts being discharged from the mouth of the "loaded" *Microstoma.*

From these observations it appears, therefore, that *Hydra* is eaten by *Microstoma* not primarily as a food but for its nematocysts. Thus, it is further suggested that the handling of the nematocysts by *Microstoma* is done in order that the rhabdocoele may use these "stinging threads."

Discharge of Nematocysts by Microstoma a Double Process.

When one teases a *Microstoma,* that is armed with *Hydra's* nematocyst, the nematocyst in the immediate neighborhood of the stimulus oscillates to and fro at right angles to the surface of the body. If the teasing is maintained long enough, the nematocyst will be discharged at the object with which the *Microstoma*

was being touched. An observer cannot watch this double reaction on the part of the *Microstoma* when being stroked with a needle point without feeling that the first phase of reaction, viz., the oscillation of the nematocyst, is a threat and that the second phase of the reaction, viz., the discharge of the nematocyst, was an effort to use the nematocyst against the annoying object.

Microstoma Uses Its Nematocysts.

Finally we have been able to see *Microstoma* actually using the nematocysts that it had appropriated from *Hydra*.

October 13, 1917, the senior author placed some Stenostomas in a hanging drop with a *Microstoma* that had nematocysts. One of the Stenostomas was at once stung along its side. The wound caused the Stenostoma's body to rupture. After the *Microstoma* had thus wounded the victim, it at once swallowed it. Mr. Conway Zirkle saw this same *Microstoma* strike another *Stenostoma* in such fashion as to cause it to bend its body near the middle at right angles and to remain quiet in this contour until the *Microstoma* fell to ingesting it. In neither of these observations were the actual nematocysts seen entering or having entered the *Stenostoma*.

But on September 9, 1917, the senior author placed a large *Microstoma*, containing nematocysts, in a hanging drop of water with three very large dividing Stenostomas. The head of one of these Stenostomas came in contact with the right side of the *Microstoma's* head. Forthwith the *Stenostoma* contracted violently. From a wound at the tip of the body the mesenchyme oozed. As this material ran out of the body, it dragged with it two nematocysts that had their filaments and barbs ejected. The *Stenostoma's* body also developed a small blister to the left of the ruptured region of the epidermis. The *Microstoma* left the *Stenostoma*, which no longer moved from place to place, and later came back and attempted to ingest the *Stenostoma* as it lay struggling as though suffering from its wound.

September 19, 1917, the senior author placed a *Microstoma*, that had nematocysts, in a hanging drop of water with a single dividing *Stenostoma*. Eight times these two animals collided.

Sometimes these collisions caused the one animal to glide down along the side of the other; at other times the collisions would be head on. In each case the collisions were so evident as to cause a shunting of one or the other specimen. At 11.30 A.M. while *Microstoma* lay quiet, *Stenostoma* made a contact with *Microstoma* and stroked the entire left side of the *Microstoma* with its right ciliated pit. When *Stenostoma's* head had come to be in contact with the posterior end of *Microstoma,* the latter discharged two nematocysts into the region of *Stenostoma's* right ciliated pit. At once a rupture took place in the *Stenostoma's* epidermis and from this wound granular material oozed. Despite this breaking of the *Stenostoma's* body in the region in which it had been stung, the *Stenostoma* was anchored to *Microstoma* by the nematocysts, the poison sacs of which were yet held within the *Microstoma.* The two animals were anchored thus long enough for me to call Dr. I. F. Lewis to my side and make a demonstration of the situation to him. Soon after this demonstration was made, the two poison sacs left the *Microstoma's* body and the latter swam away. The *Stenostoma,* which up to the time it was stung had been incessantly active, lay quite inactive for ten minutes with two nematocysts hanging from the wound on the right side of its anterior end. After that it gradually recovered itself.

It has thus been demonstrated that *Microstoma* uses the nematocysts which it takes from the *Hydra.*

Summary.

1. *Microstoma* manipulates the nematocysts of *Hydra* either (*a*) as a means of eliminating indigestible parts of its food, or (*b*) a means of defending itself.

2. The fact that the nematocysts are distributed uniformly over the surface of *Microstoma* within 12 hours after a *Hydra* has been ingested, suggests that this is not a process of elimination, (*a*), but is done in order that the nematocysts may be used, (*b*).

3. The fact that the nematocysts are retained indefinitely suggests that they have been taken up and retained for use.

4. The fact that this method of handling the nematocysts demands considerable work on the part of the endodermal cells and, in each case of a discharged nematocyst, the death of a cnidophage or mesodermal cell further suggests that it is all done with reference to use.

5. *Microstoma* incurs danger in attacking *Hydra;* for it is frequently stung and sometimes killed by the polyp. This too suggests that the *Microstoma* attacks *Hydra* for some peculiar end.

6. The *Microstoma* is able to draw nematocysts with which *Hydra* has stung it from its body, without suffering a rupture as does *Stenostoma.*

7. *Microstoma* with a few nematocysts attacks *Hydra* within a little time.

8. *Microstoma* having many nematocysts does not readily attack *Hydra.* The contrast between 7 and 8 suggests that *Microstoma* attacks *Hydra* not for food; but for its nematocysts.

9. The alimentary canal of a recently fed *Microstoma,* that has few nematocysts, may egest the "flesh" of *Hydra* and retain its nematocysts.

10. The alimentary canal of a *Microstoma* that has many nematocysts and is hungry, may egest the nematocysts of *Hydra* and retain the "flesh" of the polyp. The contrast between 9 and 10 suggests that *Microstoma* seeks primarily the nematocysts of *Hydra.*

11. The discharge of the nematocysts is a double process, involving (1) an oscillation to and fro of the nematocyst within its cnidophage, and (2) the actual discharge. The first phase may be carried on without being followed by the second phase. Neither of these has ever been seen except when some active body, like the experimenter's needle or an animal has brushed along the surface of *Microstoma.* This, too, suggests that the nematocysts are for use.

12. Finally *Microstoma* actually stings and paralyses other animals with the nematocysts it has appropriated from Hydras that it has eaten.

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Endophage = invading all of the vacuole — described by Kepner '11.

EXPLANATION OF FIGURES.

PLATE I.

FIG. 1. A *Microstoma* slowly moving amid the bases of the tentacles of *Hydra*. × 50.

FIG. 2. In response to the presence of *Microstoma* the *Hydra* has spread its expanding peristome along ventral surface of *Microstoma*. × 50.

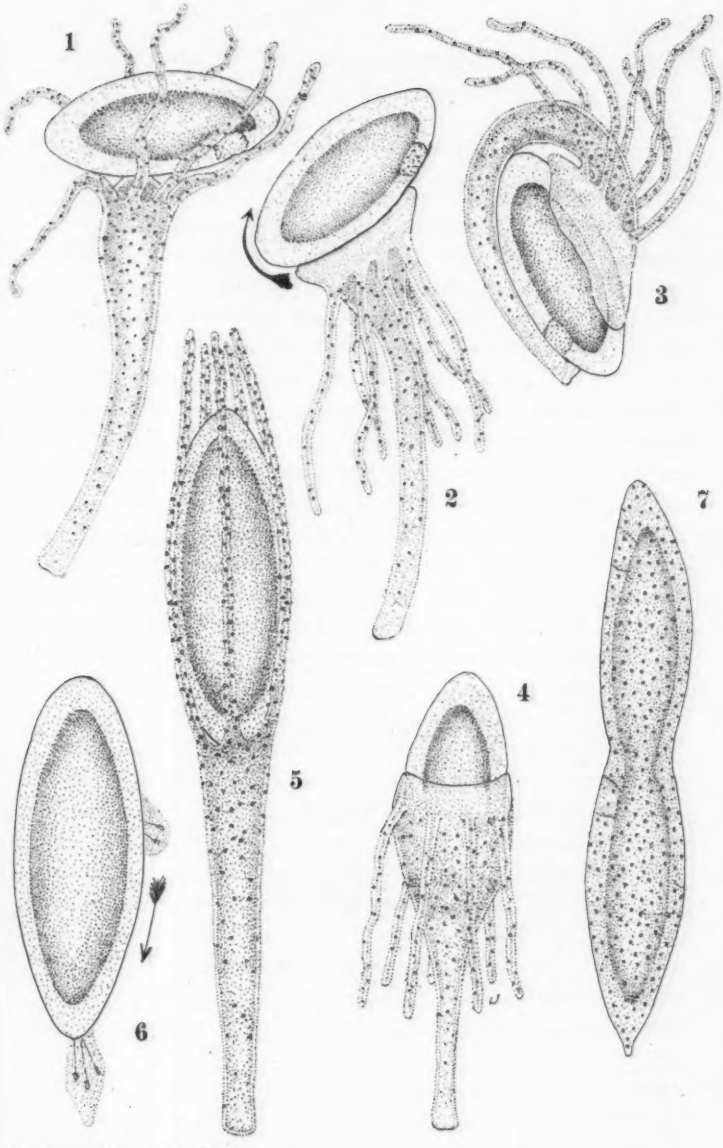
FIG. 3. The expanding peristome has glided posteriorily and dorsally, in direction indicated by arrow in Fig. 2, over surface of *Microstoma* as the *Hydra* flexed its body and pressed the *Microstoma* between its greatly expanded peristome and its bent body. From this embrace the *Microstoma* escaped. × 50.

FIG. 4. Soon after this escape the *Microstoma* returned to be captured by the tentacles of *Hydra*. After the inception of ingestion the tentacles of *Hydra* were bent back so as to lie almost parallel to the axis of the polyp. The ingestion was a slow process. × 50.

FIG. 5. Shows the manner in which a *Microstoma* was held by a *Hydra*. The *Microstoma* slowly glided out of this embrace between the ends of the tentacles. × 50.

FIG. 6. When the *Microstoma* had escaped it showed two wounded regions —one bearing two nematocysts and the other three nematocysts. These nematocysts were at first embedded so that only their poison-sacs projected from the surface of the *Microstoma*. Very early a mass of mucus formed in each wounded region. The lateral mass moved posteriorly (as shown by arrow) and fused with the posterior one. As these masses of mucus grew in length, they dragged the five stinging threads from the body of the *Microstoma*. The combined mass of mucus, containing five nematocysts, eventually was cast off. After this the *Microstoma* moved about normally. × 50.

FIG. 7. Shows a dividing *Microstoma* with a maximum charge of nematocysts at its surface. When the nematocysts are this frequent the *Microstoma* is said to be "loaded." × 50.



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SOME EFFECTS OF THE LOWER ALCOHOLS ON *PARAMECIUM*.[1]

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General—Investigators of the protozoa do not agree as to the influence of abnormal environment. For example, Matheny (1910) states that alcohol in doses of two per cent. or less "has no effect whatever" on *Paramecium*, while Calkins and Lieb (1902), and Woodruff (1908), working with doses many times more dilute report marked, but dissimilar effects.

In the present studies considerable variation in the deportment of individual paramecia from a given clone was noted, which indicates that some of the factors of error in the quantitative study of *Paramecium* are obscure, and not easy of control. As Towle (1904) observes, "The sensitiveness of paramecia for different substances varies without apparent regularity." Nevertheless it was found possible in the following experiments to obtain results of significance by counting great numbers of organisms, observing strict chemical cleanliness, and confining most of the experiments to dormant cultures of pure stocks.

Cultures—Pure lines of *Paramecium caudatum* and *Paramecium aurelia* were cultivated in battery jar infusions consisting of about 25 grams of timothy hay per liter of spring water. These were twice boiled to insure the destruction of rotifers. After about a month from the date of preparation the cultures entered upon a prolonged stage of dormancy during which little detectable change occurred until starvation was evidenced by an abrupt decline. Except where otherwise noted, only organisms from the dormant cultures were studied.

No attempt was made at bacterial control. However, in one culture a mixture of *B. lactis aerogenes* and a bacillus of the

[1] Abbreviated excerpts from an essay presented to The Johns Hopkins University in conformity with the requirements for the degree of Master of Arts (Bills, 1923*a*). A previous publication (Bills, 1923*b*) containing other excerpts should be consulted.

aquatilis group gained a long-enduring ascendency over all other bacterial forms.[2] As this culture supported the finest growth of paramecia that I have ever seen, it is interesting to note that Hargitt and Fray (1917) and Phillips (1922) maintain that simple bacterial mixtures do *not* provide as good a food for *Paramecium* as the usual complex natural mixtures.

Alcohols—The six simplest monatomic alcohols were employed: Methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, and *i*-butyl. All were of good purity. Dilutions in spring water were prepared volumetrically, with a micro-burette, a fresh solution being employed for each observation.

The Effect of Lethal Concentrations of the Alcohols—One clone of *P. aurelia* and two of *P. caudatum* were treated with such strengths of the four normal alcohols as sufficed to kill them in from 30 seconds to 30 minutes. The strongest concentration used was 15 per cent methyl alcohol; the weakest was 0.8 per cent butyl. All other strengths were intermediate. In about 300 individuals the process of dying was observed under a magnification of 700 diameters.

Wide variations disregarded, the phenomena usually observed follow in order: Incoördination and inactivation of body cilia; discharge of trichocysts; arrest of contractile vacuoles; modification of cyclosis in course and diminution in rate; bending of body to a crescent; convulsive rearrangement in posterior part, producing "Indian club" shape; arrest of undulating membrane in gullet; and at death, change in appearance of protoplasm to opaque and yellowish, cessation of Brownian movement, occasional formation of blisters by elevation of cuticle, and sometimes rupture of ectoplasm with discharge of endoplasm into the blisters.

Three points in blister formation warrant further mention: (1) The lower alcohols give rise to a *few* blisters which grow *rapidly*, whereas the higher ones result in *many* blisters which grow *slowly*.

(2) The existence of "susceptibility gradients" in *Paramecium* is nicely demonstrated in blister formation. Blisters

I am much indebted to Dr. Percy D. Meader, of the School of Hygiene and Public Health, for the bacteriological examination of this unusual culture.

rarely, if ever, form in the oral groove, and this region is generally the last to exhibit the signs of death. But the anterior end is the most susceptible to blister formation, and it is there that the ectoplasm most frequently breaks. Furthermore, the aboral side is more susceptible than the oral, being nearly as delicate as the anterior end, in regard to both blister formation and ectoplasmic rupture. In this connection it is interesting to recall the work of Child (1914) who demonstrated in *Paramecium* anterior hypersensitiveness to cyanide.

(3) When the granules of the seemingly still living protoplasm are discharged thru the ectoplasm into a blister they do not behave precisely like free particles in a liquid; they keep together in globular masses, or in thread-like protrusions. Sometimes they may become differentiated even more distinctly from the still hyaline portion of the blister by forming a new superficial film. These observations are in accord with the researches of Seifriz (1921), who noted the tendency of living protoplasm to remain immiscible with water, and "to form, almost instantly, a membrane on its surface."

Alcohol and Resistance to Starvation—None of the earlier studies on the influence of alcohol on *Paramecium* appears to have considered the effect on starving cultures. In attacking this problem cultures were prepared by adding one volume of dormant stock culture to one volume of an alcohol of twice the desired strength. Such mixtures were apportioned in 25 cc. fractions to about 100 Stender dishes of 30 cc. capacity. Most of the dishes were kept at room temperature, and the covers removed only when observations were made at various intervals. A few of the cultures were temperature-controlled.

By a method described at length in my original essay (Bills, 1923*a*) determinations were made on the maintenance of the alcoholic content of these cultures. It was found that in spite of the closely fitting covers on the dishes the alcoholic content diminished at the rate of 21 per cent. of the original amount in five days, and 56 per cent. in 31 days, these values including loss by consumption as well as loss by evaporation. Both values are averages of 45 cultures containing 1.25 per cent. ethyl alcohol.

Three sets of observations were made. The results are recorded in Tables I., II., and III. Table I. is a record of the ac-

TABLE I.

A RECORD OF THE ACTIVITY, SIZE, AND POPULATION OF CULTURES FROM CLONE 10, UNDER THE INFLUENCE OF 1.0 PER CENT. METHYL, 0.8 PER CENT. ETHYL, 0.4 PER CENT. PROPYL, AND 0.2 PER CENT. BUTYL ALCOHOLS.

Alcohol.	Exposure.	Activity.	Size.	Population.
Methyl ..	24 Hours	Normal	Decreased	
Ethyl....		Increased	Normal	
Propyl...		Normal	Normal	
Butyl....		Increased	Normal	
Methyl ..	64 Hours	Normal	Normal	Distributed throughout culture
Ethyl....		Normal	Normal	Distributed throughout culture
Propyl...		Normal	Normal	Aggregated in dense masses
Butyl....		Normal	Normal	Distributed throughout culture
Methyl ..	91 Hours	Decreased	Decreased	
Ethyl....		Normal	Normal	
Propyl...		Decreased	Decreased	
Butyl....		Normal	Normal	
Methyl ..	25 Days		Decreased	Very few
Ethyl....			Normal	Few
Propyl...			Normal	Extremely numerous
Butyl....			Normal	Extremely numerous
Methyl ..	53 Days	Decreased		Few
Ethyl....		Decreased		Very numerous
Propyl...		Decreased		Numerous
Butyl....		Decreased		Numerous
Methyl ..	120 Days			Extinct
Ethyl....				Very few
Propyl...				Extinct
Butyl....				Extinct

tivity, size, and endurance of organisms from *Paramecium caudatum,* Clone 10 exposed for varying lengths of time to the first four normal primary alcohols in the following concentrations: 1.0 per cent. methyl; 0.8 per cent. ethyl; 0.4 per cent. propyl; 0.2 per cent. butyl. Table II. is a similar record of five discrete experiments on Clone 10 showing the difference between alchoholized and normal cultures. Controls consisting of culture diluted with an equal volume of spring water were used in this series. All the treated cultures contained 1.0 per cent. methyl alcohol. Table III. is a comprehensive record of seven cultures of paramecia,

each of which was treated with six different alcohols, and observed as to population in comparison with untreated, undiluted, controls at intervals up to two months from the time of preparation. The concentrations of methyl and *n*-propyl alcohols seem to have been a little high for some of the cultures. The alcoholic content was as follows: 2 per cent. methyl; 1 per cent. ethyl; 1/2 per cent. *n*-propyl; 1/4 per cent. *n*-butyl; 3/4 per cent. *i*-propyl; 3/8 per cent. *i*-butyl.

TABLE II.

RECORDS OF FIVE DISCRETE EXPERIMENTS ON CLONE 10 IN 1.0 PER CENT. METHYL ALCOHOL, SHOWING THE DIFFERENCES BETWEEN ALCOHOLIZED AND CONTROL CULTURES AFTER DIFFERENT PERIODS OF TIME.

1. Time, 16 days. Temperature maintained at 27.5°.
   Treated culture flourishing.
   Control beginning to starve.
2. Time, 30 days. Room temperature. Covers sealed with vaseline.
   Treated culture contains many large, slow-moving animals.
   Control died of starvation.
3. Time, 30 days. Temperature maintained at 25°.
   Treated culture contains many paramecia of almost normal size, but much vacuolated and very slow-moving.
   Control died of starvation.
4. Time, 30 days. Temperature maintained at 35°.
   Treated culture contains many small, active paramecia.
   Control died of starvation.
5. Time, 50 days. Room temperature.
   Treated culture contains many large, active, slightly vacuolated paramecia.
   Control died of starvation.

Inspection of the tables reveals that all alcohols have a similar influence on starving cultures. Not only do all of them postpone the advent of starvation, but they may even restore severely starved cultures to their former prosperity. This fact should not be taken to indicate that alcohols function *directly* as food for *Paramecium*, as they appear to do for green algæ (Moore and Webster, 1920). In the present case their mode of action is obscure. In activity the alcoholized paramecia remain normal, increase, or decrease; and in size they remain normal, or decrease—conditions attributable quite as well to nutritional as to pharmacological influence.

TABLE III.

A POPULATION RECORD OF SEVEN CULTURES EXPOSED, WITH CONTROLS, TO SIX ALCOHOLS FOR DIFFERENT PERIODS OF TIME.

The alcoholic strengths were: 2 per cent. methyl; 1 per cent. ethyl; ½ per cent. *n*-propyl; ¼ per cent. *n*-butyl; ¾ per cent. *i*-propyl; ⅜ per cent. *i*-butyl.

	Alcohol.	Population at Time of Prep.	Population 4 Days Later.	Population 30 Days Later.	Population 60 Days Later.
P. caudatum Clone 10	Control	Pale, odorless, nearly extinct from starvation.	Nearly extinct	Nearly extinct	Extinct
	Methyl		Extinct	.....	.....
	Ethyl		Multiplying	Very numerous	Excellent
	n-Propyl		Extinct	.....	.....
	n-Butyl		Slight increase	Very numerous	Numerous thin
	i-Propyl		Nearly extinct	Numerous	Extinct
	i-Butyl		Extinct	.....	.....
P. caudatum Clone 10	Control	Pale, putrid, extremely numerous. Some aggregates of paramecia have been broken up.	Excellent	Very numerous	Very few thin
	Methyl		Excellent thin	Numerous fat	Extinct
	Ethyl		Excellent	Excellent	Numerous small
	n-Propyl		Extinct	.....	.....
	n-Butyl		Excellent	Very numerous	Numerous
	i-Propyl		Numerous	Numerous	Extinct
	i-Butyl		Excellent	Numerous	Few but good
P. caudatum Clone 8	Control	Pale, odorless, dormant. Will soon be starving.	Excellent	Very few	Extinct
	Methyl		Nearly extinct	Extinct	.....
	Ethyl		Excellent	Very few	Very few
	n-Propyl		Extinct	.....	.....
	n-Butyl		Excellent	Few	Extinct
	i-Propyl		Excellent	Few	Extinct
	i-Butyl		Excellent	Very numerous	Nearly extinct
P. aurelia Clone 11	Control	Colorless, odorless, few paramecia in starvation.	Excellent	Few	Extinct
	Methyl		Few	Very few	Numerous good
	Ethyl		Excellent	Nearly extinct	Extinct
	n-Propyl		Extinct	.....	.....
	n-Butyl		Numerous	Nearly extinct	Extinct
	i-Propyl		Excellent	Extinct	.....
	i-Butyl		Very few	Extinct	.....
P. aurelia Clone 7	Control	Pale, odorless, dormant.	Numerous	Nearly extinct	Extinct
	Methyl		Few	Extinct	.....
	Ethyl		Numerous	Numerous	Accident
	n-Propyl		Extinct	.....	.....
	n-Butyl		Multiplying	Very numerous	Very few
	i-Propyl		Numerous	Few	Extinct
	i-Butyl		Numerous	Extinct	.....
P. aurelia Clone 4	Control	Pale, odorless. Few paramecia, somewhat starved.	Numerous	Very numerous	Few thin
	Methyl		Numerous	Very numerous	Few good
	Ethyl		Multiplying	Nearly extinct	Few thin
	n-Propyl		Extinct	.....	.....
	n-Butyl		Numerous	Nearly extinct	Numerous
	i-Propyl		Numerous	Numerous	Accident
	i-Butyl		Very few	Numerous	Numerous
P. caudatum Wild culture	Control	Colorless, putrid. The aggregates of paramecia have been well broken up. Extremely numerous.	Extinct	.....	.....
	Methyl		Extinct	.....	.....
	Ethyl		Few	Excellent	Numerous thin
	n-Propyl		Few	Very numerous	Numerous small
	n-Butyl		Very numerous	Few thin	Nearly extinct
	i-Propyl		Few	Very few	Few thin
	i-Butyl		Numerous	Excellent	Numerous thin

The Influence of Temperature on the Susceptibility of Paramecium to Ethyl Alcohol.—Within reasonable limits of constancy a given concentration of a given alcohol will narcotize a definite per cent. of the organisms in a particular culture in one hour. This fact makes it possible to compare quantitatively the narcotic action of various alcohols, and to study the modifying influence of physical conditions on the effects of a particular alcohol. The method devised for counting the paramecia "narcotized" and those "unaffected" is elsewhere described (Bills, 1923*b*).

In the present experiment counts were made at widely different temperatures—8° and 25°. *Paramecium caudatum,* Clone 10, and 3.0 per cent. ethyl alcohol were used, and all observations made in duplicate. From the data presented in Table IV. it appears that the per cent. of the paramecia narcotized at 8° does not differ significantly from the per cent. at 25°. It seems improbable (though of course possible) that intermediate temperatures would show any markedly different values.

TABLE IV.

SHOWING THE INFLUENCE OF TEMPERATURE ON THE SUSCEPTIBILITY OF PARAMECIA TO ETHYL ALCOHOL.

The experiments were conducted in darkness.

Temperature.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.
8°	50	793	5.9
8°	43	473	8.3
25°	47	568	7.6
25°	32	358	8.2

Average per cent. narcotized at 8° = 7.1.
Average per cent. narcotized at 25° = 7.9.

The Influence of Light on the Susceptibility of Paramecium to Ethyl Alcohol.—Pairs of burettes containing paramecia of Clone 10, with and without 3.0 per cent. ethyl alcohol were kept for one hour in strong, but diffuse, northern, daylight, or in direct, brilliant, sunlight in the middle of April. The direct light passed obliquely through the thin glass walls of the burettes. In all experiments the temperature was between 21° and 23°.

The data are presented in Table V. This table shows that direct sunlight inactivated in one hour 28 per cent. of the organ-

isms in plain spring water, but that under like conditions, except that 3.0 per cent. ethyl alcohol was present, the sunlight inactivated 42 per cent. The experiments were repeated in diffuse daylight, and not one individual was inactivated in the absence of alcohol, while with 3.0 per cent. alcohol 8.9 per cent. were affected—a figure not significantly different from the values got in the temperature experiments which were made in darkness.

TABLE V.

SHOWING THE INFLUENCE OF LIGHT ON THE SUSCEPTIBILITY OF PARAMECIA TO ETHYL ALCOHOL.

Light.	Per Cent. Alcohol.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.	Average.
Diffuse daylight....	0.0	0	1361	0.0	0.0
Diffuse daylight....	3.0	213	1641	12	
Diffuse daylight....	3.0	28	454	5.8	8.9
Direct sunlight.....	0.0	558	1432	28	
Direct sunlight.....	0.0	451	1150	28	28
Direct sunlight.....	3.0	888	914	49	
Direct sunlight.....	3.0	726	1306	35	42

The Combined Effect of Preliminary Aëration and Agitation of a Paramecium Culture on its Subsequent Susceptibility to an Alcohol.—Aëration was effected by agitating for two minutes some paramecia of Clone 10 in 1.6 per cent. of *i*-propyl alcohol. This alcohol was chosen because of the fine froth produced when cultures containing it are violently shaken. The results presented in Table VI. show that the aërated paramecia are decidedly less susceptible than normal controls. Of the agitated organisms 17 per cent. were narcotized, whereas 32 per cent. were narcotized in the non-aërated control culture.

TABLE VI.

SHOWING THE INFLUENCE OF AËRATION AND AGITATION ON THE SUSCEPTIBILITY OF PARAMECIA TO 1.6 PER CENT. *i*-PROPYL ALCOHOL.

Treatment of Culture.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.
Agitated and aërated....	239	1139	17
Normal control.....	682	1460	32

The Question of the Adaptation of Parmecium to Alcohol.— How does a preliminary exposure of *Paramecium* to a low concentration of ethyl alcohol affect the subsequent resistance to a stronger dose of ethyl alcohol, and of its homologues?

Towle (1904), working with electrolytes and simple organic compounds, concluded that "paramecia become readily habituated to solutions in strengths which are not soon fatal." Daniel (1908) found that paramecia when transferred *gradually* into distilled water become adjusted to this otherwise deadly substance. Estabrook (1910) developed in *Paramecium* a temporarily increased tolerance for strong doses of sodium chloride. Neuschlosz (1921*a*) found that *Paramecium* can develop a high resistance to dyes of the thiazin, benzidin, and triphenylmethane series. Neuschlosz later (1921*b*) reported that paramecia acclimatized to trivalent arsenic are at the same time resistant to trivalent antimony. Woodruff (1908) observed that alcoholized paramecia become more sensitive to copper sulphate. Their behavior toward a stronger dose of *alcohol* was not recorded. A case of adaptation in *Spirostomum* and *Stentor* reported by Daniel (1909) is of special interest, inasmuch as the method of experimentation is essentially identical with my method on *Paramecium;* the results, however, being different from mine. Daniel claims that he sometimes produced in these protozoa a slight adaptation to ethyl alcohol, but that this was accompanied by an increased susceptibility to methyl alcohol.

My experiments were made as follows: To 10 cc. of the Clone 10 culture taken from near the surface 10 cc. of 2.0 per cent. ethyl alcohol was added, making a 1.0 per cent. solution of alcohol. This mixture was put into a 30 cc. Stender dish and kept at approximately 24° for three days. At the end of this period the paramecia were observed to be distributed thruout the medium, appearing healthy, and distinctly more active than the controls altho possibly somewhat thinner. They were then exposed for one hour to each of the six alcohols in the concentrations indicated in Table VII., using quantities large enough to eliminate practically all error resulting from the presence of the original ethyl alcohol (see Bills, 1923*a*).

The results obtained are presented in Table VII. In this

table the values given for the untreated controls are interpolated averages obtained for the six alcohols in a series of experiments elsewhere described (Bills, 1923*b*). They were made a few days before the present experiment was performed, during a period of extended constancy in the cultures. Therefore, these values are admissible for comparison here, and are probably more nearly accurate than single observations would have been.

From Table VII. it is clear that the three-day exposure to 1.0 per cent. ethyl alcohol increased the susceptibility of the paramecia to a narcotizing concentration of ethyl alcohol; and, similarly, to each of the other five alcohols. In other words, paramecia are not acclimatized to ethyl alcohol under the conditions of this experiment. Unlike Daniel's spirostoma and stentors which under similar conditions became more resistant to ethyl alcohol, paramecia became more susceptible to *all* alcohols.

TABLE VII.

SHOWING THE EFFECT OF EXPOSURE TO ALCOHOL ON THE ACTION OF ALCOHOLS ON *Paramecium*.

Period of Acclimatization to 1.0 Per Cent. Ethyl Alcohol.	Narcotizing Alcohols.	Per Cent. of Treated Paramecia Narcotized.	Per Cent. of Untreated Paramecia Narcotized.
72 hours.....	Methyl, 5.0%	Mostly disintegrated	30
72 hours.....	Ethyl, 3.3%	Many disintegrated	24
77 hours.....	*n*-Propyl, 0.9%	35	31
77 hours.....	*n*-Butyl, 0.5%	44	31
79 hours.....	*i*-Propyl, 1.6%	55	29
79 hours.....	*i*-Butyl, 0.4%	54	35

I wish to express my appreciation of the interest and guidance given me in the course of these experiments by Professor S. O. Mast and Professor H. S. Jennings; and my thanks to many other persons for their assistance in many ways.

SUMMARY.

1. A mixture of *B. lactis aerogenes* and *B. aquatilis* (sp. ?) constitutes the best food found for *Paramecium*.

2. When paramecia are exposed to an alcohol in sufficient strength they are at first incoördinated in movement and then inactivated. Later toxic effects are manifested by marked in-

ternal changes, formation of "blisters" by elevation of cuticle, rupture of ectoplasm, and death.

3. The anterior end of *Paramecium* is more susceptible to alcohol than the posterior end, and the aboral side more than the oral.

4. Indirect daylight has no perceptible effect on normal or alcoholized paramecia, but direct sunlight inactivates them; this effect is augmented in the presence of alcohol.

5. Change in temperature over a wide range has no appreciable effect on the susceptibility of paramecia to alcohol.

6. Aëration and agitation of a *Paramecium* culture renders the paramecia much less susceptible to alcohol.

7. Paramecia in a given solution without food live longer with alcohol than without; starving cultures can even be restored to prosperity by the addition of suitable amounts of any alcohol.

8. Exposure of paramecia to weak ethyl alcohol increases their susceptibility to a stronger dose of ethyl alcohol, and to five other alcohols.

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